II. REMARKS

Claims 1-88 were pending. Claims 21, 51 and 79 have been canceled without prejudice or disclaimer and claims 1, 2,4, 9, 14,18-20, 23, 24, 28, 31,32, 34, 39, 44, 48, 49, 50, 52-54, 58, 61, 62, 64 ,69, 73, 76-78, 80-82, and 86 have been amended. Support for the amendments can be found throughout the original claims and specification as filed. For example, the term "engineered" can be found, for example, in the section entitled "Design of ZFPs" from page 21 through page 25, particularly page 21, lines 10-11. Engineered ZFPs include those that are designed and selected, for example, as described on page 21, line 21-31; page 24, lines 23-33; and page 28, lines 3-32. No new matter has been added as a result of these amendments and entry thereof is respectfully requested. Applicants reserve the right to file a continuation or divisional directed to the subject matter of the canceled claims at any time during the pendency of this application.

35 U.S.C. § 112, First Paragraph

In the parent case, the Office had rejected the claims similar to those recited herein on the grounds that Applicants' disclosure enabled the claimed methods only in an isolated cell. (See, Office Action mailed May 14, 2001 in parent application 09/229,037). Applicants submit that the evidence of record establishes that the claims as pending are fully enabled by the specification as filed.

It is well-settled that the enablement requirement is satisfied if the applicant's specification teaches one of skill in the art how to make and use the claimed invention without undue experimentation. *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988). When determining whether the amount of testing required is "undue", the courts have determined that "time and difficulty of experiments are not determinative if they are merely routine." (see, *e.g., In re Wands,* 8 USPQ2d at 1404, citing *In re Angstadt*, 190 USPQ 214 (CCPA 1976). Thus, Applicants reiterate that the test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in

the art without undue experimentation. *Ex parte Forman*, 230 USPQ 546 (P.T.O. Bd. Pat. App. & Int., 1986).

In addition, to further rebut any argument that using the claimed methods in non-isolated cell is "unpredictable" or would require "undue experimentation," Applicants submit herewith Declarations by Dr. Case and Dr. Giordano documenting that, using the methods set forth in the specification, a skilled artisan could readily practice the claimed methods *in vivo*. Thus, Dr. Giordano states:

> 4. Our results show that VEGF expression was enhanced *in vivo* in both skeletal and cardiac muscle upon injection of plasmids encoding VEGF-targeted ZFPs. .... The internal actin standard indicates that enhancement of VEGF expression in these tissues was not due to injection *per se*. These studies demonstrate that plasmid constructs encoding ZFPs regulate gene expression *in vivo*. ...
>
> 6. Thus, using the polynucleotides encoding VEGF-targeted ZFPs provided by Sangamo Biosciences, we demonstrated that various gene delivery vectors encoding these ZFPs were able to regulate expression of VEGF *in vivo*.
>
> 7. Using several different accepted model systems for angiogenesis and wound healing, our investigations also show that VEGF-targeted ZFPs can be utilized to modulate angiogenesis and thus affect a wide variety of *in vivo* conditions that are correlated with blood flow and blood delivery. More specifically, our results demonstrate that angiogenesis and related processes can be regulated by introducing gene delivery vehicles (*e.g.*, plasmids, viral constructs, *etc.*) encoding ZFPs which modulate expression of one or more VEGF genes *in vivo*. ...
>
> 9. VEGF-regulating ZFPs also augment wound healing (*e.g.*, re-epithelization, keratinocyte ingrowth) in a well-established cutaneous wound healing model ... Thus, *in vivo* activation of endogenous VEGF by targeted ZFP molecules results in faster wound healing as evidenced by increased re-epithelization and more rapid keratinocyte ingrowth. ...
>
> 11. In light of these results, I conclude that engineered ZFPs can be used to modulate expression of endogenous genes in living animals. Modulation of endogenous gene expression by engineered ZFPs can be accomplished using a variety of different zinc finger proteins and a variety of different administration modalities (*e.g.*, injection and/or topical administration into skeletal muscle, cardiac muscle and/or ear tissue using plasmid, viral or other vectors). Further, the studies reported herein indicate that modulation of endogenous gene expression by engineered ZFPs can affect conditions associated with expression

of the target gene; for example, modulation of VEGF expression affects the processes of angiogenesis and wound healing. Because ZFPs can be designed and/or selected to bind to any predetermined sequence, methods similar to those described in this declaration are equally applicable to any endogenous gene of interest.

Thus, as indicated in the attached Declarations, the teachings of the specification are more than sufficient to enable one of skill in the art to practice the claimed methods in any cell in any organism.

III. CONCLUSION

For the foregoing reasons, Applicants believe the claims are in condition for allowance and request early notification to that effect.

Respectfully submitted,

Date: Nov 14, 2001

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Version Showing Changes Made to Claims

1. (Amended) A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with [a first] the zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene [by at least about 20%].

2. (Amended) The method of claim 1, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with [a] the second zinc finger protein.

4. (Amended) The method of claim 3, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

9. (Amended) A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a regulatory domain; and

contacting a target site in the endogenous cellular gene with [a] the fusion zinc finger protein [comprising six fingers and a regulatory domain], wherein the K_d of the fusion zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene[by at least about 20%].

14. (Amended) The method of claim 1, wherein the endogenous cellular gene is [a] selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, and Her2/Neu.

18. (Amended) The method of claim 1, wherein [the method further comprises] the step of [first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide] administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

19. (Amended) The method of claim 1, wherein the [zinc finger protein is encoded by a zinc finger protein nucleic acid] nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter[, and wherein the method further comprises the step of first administering the nucleic acid to the cell in a lipid:nucleic acid complex or as naked nucleic acid].

20. (Amended) The method of claim 1, wherein [the zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell] the expression vector is a viral expression vector.

21. Canceled.

22. (Amended) The method of claim 20, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, [a DNA plasmid expression vector] or an AAV expression vector.

23. (Amended) The method of claim 20, wherein [the zinc finger protein is encoded by a nucleic acid] the promoter to which the zinc finger-encoding nucleic acid is operably linked [to] is an inducible promoter.

24. (Amended) The method of claim 20, wherein [the zinc finger protein is encoded by a] the promoter to which the zinc finger-encoding nucleic acid is operably linked is [to] a weak promoter.

28. (Amended) The method of claim 1, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription initiation site of the endogenous cellular gene.

31. (Amended) A method of activating expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with the [a first] zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene[to at least about 150%].

32. (Amended) The method of claim 31, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a

promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with [a] the second zinc finger protein.

34. (Amended) The method of claim 33, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

39. (Amended) A method of activating expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a regulatory domain; and

contacting a target site in the endogenous cellular gene with [a] the fusion zinc finger protein [comprising six fingers and a regulatory domain], wherein the K_d of the fusion zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene[to at least about 150%].

44. (Amended) The method of claim 31, wherein the endogenous cellular gene is [a] selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-R.

48. (Amended) The method of claim 31, wherein the [method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide] step of administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

49. (Amended) The method of claim 31, wherein the [zinc finger protein is encoded by a zinc finger protein nucleic acid] nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter[, and wherein the method further comprises the step of first administering the nucleic acid to the cell in a lipid:nucleic acid complex or as naked nucleic acid].

50. (Amended) The method of claim 31, wherein [the zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell] the expression vector is a viral expression vector.

51. Canceled.

52. (Amended) The method of claim 50, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, [a DNA plasmid vector] or an AAV expression vector.

53. (Amended) The method of claim 50, wherein [the zinc finger protein is encoded by a nucleic acid] the promoter to which the zinc finger-encoding nucleic acid is operably linked is [to] an inducible promoter.

54. (Amended) The method of claim 50, wherein [the zinc finger protein is encoded by a] the promoter to which the zinc finger-encoding nucleic acid is operably linked is [to] a weak promoter.

58. (Amended) The method of claim 31, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription initiation site of the endogenous cellular gene.

61. (Amended) A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with the [a first] zinc finger protein,

thereby modulating expression of the endogenous cellular gene.

62. (Amended) The method of claim 61, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with [a] the second zinc finger protein.

64. (Amended) The method of claim 63, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

69. (Amended) A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the [step] steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a

regulatory domain; and

contacting a target site in the endogenous cellular gene with [a] the fusion zinc finger protein [comprising six fingers and a regulatory domain];

thereby modulating expression of the endogenous cellular gene.

73. (Amended) The method of claim 61, wherein the endogenous cellular gene is [a] selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

76. (Amended) The method of claim 61, wherein the [method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide] step of administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

77. (Amended) The method of claim 61, wherein the [zinc finger protein is encoded by a zinc finger protein nucleic acid] nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter[, and wherein the method further comprises the step of first administering the nucleic acid to the cell in a lipid:nucleic acid complex or as naked nucleic acid].

78. (Amended) The method of claim 61, wherein [the zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell] the expression vector is a viral expression vector.

79. Canceled.

80. (Amended) The method of claim 78, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, [a DNA plasmid expression vector,] or an AAV expression vector.

81. (Amended) The method of claim 78, wherein [the zinc finger protein is encoded by a nucleic acid] the promoter to which the zinc finger-encoding nucleic acid is operably linked is [to] an inducible promoter.

82. (Amended) The method of claim 78, wherein [the zinc finger protein is encoded by a] the promoter to which the zinc finger-encoding nucleic acid is operably linked is [to] a weak promoter.

86. (Amended) The method of claim 61, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription initiation site of the endogenous cellular gene.

Currently Pending Claims

1. (Amended) A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with the zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

2. (Amended) The method of claim 1, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with the second zinc finger protein.

3. The method of claim 2, wherein the first and second target sites are adjacent.

4. (Amended) The method of claim 3, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

5. The method of claim 1, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

6. The method of claim 5, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

7. The method of claim 2, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

8. The method of claim 7, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

9. (Amended) A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a

regulatory domain; and

contacting a target site in the endogenous cellular gene with the fusion zinc finger protein, wherein the K_d of the fusion zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

10. The method of claim 1, wherein the cell is selected from the group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

11. The method of claim 10, wherein the cell is a mammalian cell.

12. The method of claim 11, wherein the cell is a human cell.

13. The method of claim 1, wherein expression of the endogenous cellular gene is inhibited by at least about 75%-100%.

14. (Amended) The method of claim 1, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, and Her2/Neu.

15. The method of claim 1, wherein the endogenous cellular gene is VEGF.

16. The method of claim 1, wherein the inhibition of gene expression prevents gene activation.

17. The method of claim 5 or claim 7, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

18. (Amended) The method of claim 1, wherein the step of administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

19. (Amended) The method of claim 1, wherein the nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter.

20. (Amended) The method of claim 1, wherein the expression vector is a viral expression vector.

21. Canceled.

22. (Amended) The method of claim 20, wherein the expression vector is a retroviral

expression vector, an adenoviral expression vector, or an AAV expression vector.

23. (Amended) The method of claim 20, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is an inducible promoter.

24. (Amended) The method of claim 20, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is a weak promoter.

25. The method of claim 1, wherein the cell comprises less than about $1.5x10^6$ copies of the zinc finger protein.

26. The method of claim 1, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

27. The method of claim 1, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

28. (Amended) The method of claim 1, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription initiation site of the endogenous cellular gene.

29. The method of claim 1, wherein the zinc finger protein comprises an SP-1 backbone.

30. The method of claim 29, wherein the zinc finger protein comprises a regulatory domain and is humanized.

31. (Amended) A method of activating expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with the zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

32. (Amended) The method of claim 31, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with the second zinc finger protein.

33. The method of claim 32, wherein the first and second target sites are adjacent.

34. (Amended) The method of claim 33, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

35. The method of claim 31, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

36. The method of claim 35, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

37. The method of claim 32, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

38. The method of claim 37, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

39. (Amended) A method of activating expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a regulatory domain; and

contacting a target site in the endogenous cellular gene with the fusion zinc finger protein, wherein the K_d of the fusion zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

40. The method of claim 31, wherein the cell is selected from the group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

41. The method of claim 40, wherein the cell is a mammalian cell.

42. The method of claim 41, wherein the cell is a human cell.

43. The method of claim 31, wherein the endogenous cellular gene is activated to at least about 200-500%.

44. (Amended) The method of claim 31, wherein the endogenous cellular gene is selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-R.

45. The method of claim 31, wherein the endogenous cellular gene is VEGF.

46. The method of claim 31, wherein the activation of gene expression prevents repression of gene expression.

47. The method of claim 35 or 37, wherein the regulatory domain is selected from the group consisting of a transcriptional activator, or a histone acetyltransferase.

48. (Amended) The method of claim 31, wherein the step of administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

49. (Amended) The method of claim 31, wherein the nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter.

50. (Amended) The method of claim 31, wherein the expression vector is a viral expression vector.

51. Canceled.

52. (Amended) The method of claim 50, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, or an AAV expression vector.

53. (Amended) The method of claim 50, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is an inducible promoter.

54. (Amended) The method of claim 50, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is a weak promoter.

55. The method of claim 31, wherein the cell comprises less than about $1.5x10^6$ copies of the zinc finger protein.

56. The method of claim 31, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

57. The method of claim 31, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

58. (Amended) The method of claim 31, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription

initiation site of the endogenous cellular gene.

59. The method of claim 1, wherein the zinc finger protein comprises an SP-1 backbone.

60. The method of claim 29, wherein the zinc finger protein comprises a regulatory domain and is humanized.

61. (Amended) A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, and wherein the nucleic acid molecule expresses the zinc finger protein in the cell; and

contacting a first target site in the endogenous cellular gene with the zinc finger protein, thereby modulating expression of the endogenous cellular gene.

62. (Amended) The method of claim 61, wherein the step of administering further comprises administering a second zinc finger protein-encoding nucleic acid operably linked to a promoter that expresses a second zinc finger protein in the cell, and wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with the second zinc finger protein.

63. The method of claim 62, wherein the first and second target sites are adjacent.

64. (Amended) The method of claim 63, wherein the first and second zinc finger proteins are covalently linked, forming a fusion protein.

65. The method of claim 61, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

66. The method of claim 65, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

67. The method of claim 62, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

68. The method of claim 67, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

69. (Amended) A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the steps of:

administering to the cell a nucleic acid molecule comprising a polynucleotide sequence which encodes an engineered fusion zinc finger protein, wherein said polynucleotide sequence is operably linked to a promoter, wherein the nucleic acid molecule expresses a fusion zinc finger protein in the cell, and wherein the fusion zinc finger protein comprises six fingers and a regulatory domain; and

contacting a target site in the endogenous cellular gene with the fusion zinc finger protein;

thereby modulating expression of the endogenous cellular gene.

70. The method of claim 61, wherein the cell is selected from the group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

71. The method of claim 70, wherein the cell is a mammalian cell.

72. The method of claim 61, wherein the cell is a human cell.

73. (Amended) The method of claim 61, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

74. The method of claim 61, wherein the endogenous cellular gene is VEGF.

75. The method of claim 65 or claim 67, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

76. (Amended) The method of claim 61, wherein the step of administering the nucleic acid molecule to the cell comprises administering the nucleic acid molecule in a lipid:nucleic acid complex or as naked nucleic acid.

77. (Amended) The method of claim 61, wherein the nucleic acid molecule is an expression vector comprising a zinc finger protein-encoding nucleic acid operably linked to a promoter.

78. (Amended) The method of claim 61, wherein the expression vector is a viral expression vector.

79. Canceled.

80. (Amended) The method of claim 78, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, or an AAV expression vector.

81. (Amended) The method of claim 78, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is an inducible promoter.

82. (Amended) The method of claim 78, wherein the promoter to which the zinc finger-encoding nucleic acid is operably linked is a weak promoter.

83. Canceled.

84. The method of claim 61, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

85. The method of claim 61, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

86. (Amended) The method of claim 61, wherein the target site is adjacent to an RNA polymerase pause site, wherein the RNA polymerase pause site is downstream of a transcription initiation site of the endogenous cellular gene.

87. The method of claim 61, wherein the zinc finger protein comprises an SP-1 backbone.

88. The method of claim 88, wherein the zinc finger protein comprises a regulatory domain and is humanized.

Marked-Up Specification Paragraphs:

On page 1, the paragraph beginning on line 7 has been changed as follows:

-- ~~This application is related to Townsend and Townsend and Crew docket number 019496-001800, USSN _____, filed January 12, 1999, herein incorporated by reference in its entirety.~~ This application is a continuation of United States Serial Number 09,229,037, filed January 12, 1999, from which priority is claimed pursuant to 35 U.S.C. § 120, and which application is incorporated herein by reference in its entirety.--

On page 21, the paragraph beginning on line 10 has been changed as follows:

--The ZFPs of the invention are engineered to recognize a selectable target site in the endogenous gene of choice. Typically, a backbone from any suitable C_2H_2 ZFP, such as SP-1, SP-1C, or ZIF268 is used as the scaffold for the engineered ZFP (*see, e.g.,* Jacobs *EMBO J.* 11:4507 (1992); Desjarlais & Berg, *PNAS* 90:2256-2260 (1993)). A number of methods can then be used to design and select a ZFP with high affinity for its target (e.g., preferably with a K_d of less than about 25 nM). As described above, a ZFP can be designed or selected to bind to any suitable target site in the target endogenous gene, with high affinity. ~~Co-pending patent application USSN _____, filed January 12, 1999 (TTC docket no. 019496-001800, herein incorporated by reference)~~ Co-owned WO 00/42219, incorporated by reference herein in its entirety, comprehensively describes methods for design, construction, and expression of ZFPs for selected target sites.--

On page 21, please replace the paragraph beginning on line 32 with the following:

--In a preferred embodiment, co-owned WO 00/42219 provides methods that select a target gene, and identify a target site within the gene containing one to six (or more) D-able sites (see definition below). Using these methods, a ZFP can then be synthesized that binds to the preselected site. These methods of target site selection are premised, in part, on the recognition that the presence of one or more D-able sites in a target segment confers the potential for higher binding affinity in a ZFP selected or designed to bind to that site relative to ZFPs that bind to target segments lacking D-able sites. Experimental evidence supporting this insight is provided in Examples 2-9 of ~~copending application USSN _____, filed January 12, 1999 (TTC docket no. 019496-001800)~~ co-owned WO 00/42219.--

On page 22, please replace the paragraph beginning on line 9 with the following:

A D-able site or subsite is a region of a target site that allows an appropriately designed single zinc finger to bind to four bases rather than three of the target site. Such a zinc finger binds to a triplet of bases on one strand of a double-stranded target segment (target strand) and a fourth base on the other strand (*see* Figure 1 of ~~copending application USSN _____, filed January 12, 1999 (TTC docket no. 019496-001800)~~ co-owned WO 00/42219). Binding of a single zinc finger to a four base target segment imposes constraints both on the sequence of the target strand and on the amino acid sequence of the zinc finger. The target site with the target

strand should include the "D-able" site motif 5' NNGK 3', in which N and K are convention IUPAC-IUB ambiguity codes. A zinc finger for binding to such a site should include an arginine residue at position -1 and an aspartic acid, (or less preferably a glutamic acid) at position +2. The arginine residues at position -1 interacts with the G residue in the D-able site. The aspartic acid (or glutamic acid) residue at position +2 of the zinc finger interacts with the opposite strand base complementary tot he K base in the D-able site. It is the interaction between aspartic acid (symbol D) and the opposite strand base (fourth base) that confers the name D-able site. AS is apparent from the D-able site formula, there are two subtypes of D-able sites; 5' NNGG 3' and 5' NNGT 3'. For the former site, the aspartic acid or glutamic acid at position +2 of a zinc finger interacts with a C in the opposite strand to the D-able site. In the latter site, the aspartic acid or glutamic acid at position +2 of a zinc finger interacts with an A in the opposite strand of the D-able site. In general, NNGG is preferred over NNGT.--

On page 27, please replace the paragraph beginning on line 8 with the following:

-- The biochemical properties of the purified proteins, e.g., K_d, can be characterized by any suitable assay. In one embodiment, K_d is characterized via electrophoretic mobility shift assays ("EMSA") (Buratowski & Chodosh, *in Current Protocols in Molecular Biology* pp. 12.2.1.-12.2.7 (Ausubel ed., 1996); *see also* U.S. Patent No. 5,789,538; ~~USSN _____, filed January 12, 1999 (TTC docket no. 019496-001800), herein incorpoated by reference, and Example 1 of~~ co-owned WO 00/42219 and Example 1, *infra.* Affinity is measured by titrating purified protein against a low amount of labeled double-stranded oligonucleotide target. The target comprises the nature binding site sequence (9 or 18 bp) flanked by the 3 bp found in the natural sequence. External to the binding site plus flanking sequence is a constant sequence. The annealed oligonucleotide targets possess a 1 bp 5' overhang which allows for efficient labeling of the target with T4 phage polynucleotide kinase. For the assay the target is added at a concentration of 40 nM or lower (the actual concentration is kept at least 10-fold lower than the lowest protein dilution) and the reaction is allowed to equilibrate for at least 45 min. In addition the reaction mixture also contains 10 mM Tris (pH 7.5), 100 mM KCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 5 mM DTT, 10% glycerol, 0.02% BSA (poly (dIdC) or (dAdT) (Pharamacia) can also be added at 10-100 μg/μl).--